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On the effect of temperature on the insertion of zinc into hydroxyapatite

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Abstract:

Rietveld analysis on X-ray powder diffraction patterns recorded from 28 hydroxyapatite samples containing various amount of zinc (0, 1.6, 3.2 and 6.1 wt. Zn %) and heat treated at various temperatures (between 500°C and 1100°C) have allowed to finely characterize the Zn insertion mechanism into the HAp crystal structure. The formation of Zn-doped HAp was achieved above 900°C only. Zn-doped HAp has the $\text{Ca}_{10}\text{Zn}_x(\text{PO}_4)_6(\text{OH})_{2-2x}\text{O}_{2x}$ ($0 < x \leq 0.25$) chemical composition with a constant Ca/P ratio of 1.67 due to the insertion mechanism into the hexagonal channel (partial occupancy of the $2b$ Wyckoff site with formation of linear O-Zn-O entities). Samples heat treated at 500 °C were almost single phase, HAp did not incorporate Zn and about half of the Zn atoms incorporated during the synthesis are not observable by X-ray powder diffraction (contained in an amorphous compound or physisorbed at the HAp surface). The reversible formation of Zn-doped β -TCP phase was observed at 600°C, reached its maximum content at 900°C and almost vanished at 1100°C. The results presented here strengthen the recently described mechanism of Zn insertion in the interstitial $2b$ Wyckoff position of the HAp structure, and explain the origin of the contradictory reports in the corresponding literature.

1- Introduction

Apatites $\text{Ca}_{10}(\text{PO}_4)_6(\text{F}, \text{Cl}, \text{OH})_2$, are a complex and diverse class of materials [1] which have gained increasing importance due to their biological role. One of the main constituents of bones and hard tissues in mammals is a calcium phosphate mineral whose structure closely resembles hydroxyapatite (HAp), $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. The so-called biological apatite refers to poorly crystallized non-stoichiometric carbonate-containing HAp. The inorganic content varies from 65% in bones to 90% in dental enamel [2].

Because biological apatites are formed in biological conditions, they usually contain a large variety of doping elements that can also have some specific biological properties (F, Si, Sr, Mg,...). The important role of zinc has been put forward in the recent literature [3-6]. In effect, uptake and release of Zn in the body are strongly mediated by the bone reservoir, where the Zn content ranges from 125 to 250 ppm (against 28-33 ppm for whole body) [6]. It has been demonstrated that zinc has a stimulatory effect on bone formation and mineralization in vivo and in vitro [7,8], and that Zn incorporation into implants promotes bone formation around the material [9-11], improves biological properties [9,12] decreases the inflammatory response [13,14], and has an antibacterial effect [15].

In order to understand the mechanisms of incorporation of doping elements in HAp, and to correctly characterize natural and/or pathological nanocrystalline multi-substituted apatite materials, it is of great importance to perform detailed structural characterizations of substituted synthetic HAp. Our previous study has established the Zn location into the HAp structure [16]. For high synthesis temperatures (1100°C), Zn^{2+} cations are inserted along the hexagonal channel of HAp structure at the 2b Wyckoff site forming O-Zn-O linear entities. Zn^{2+} leads to an insertion solid solution with general chemical formula $\text{Ca}_{10}\text{Zn}_x(\text{PO}_4)_6(\text{OH})_{2-2x}\text{O}_{2x}$, contrary to β -tricalcium phosphate (β -TCP, $\beta\text{-Ca}_3(\text{PO}_4)_2$) that realizes a substitution solid solution with chemical formula $\text{Ca}_{3-x}\text{Zn}_x(\text{PO}_4)_2$. The present study aims to enlarge the

investigation of the Zn^{2+} incorporation in HAp versus the temperature (heat treatment from 500 °C to 1100 °C). This study was motivated by the contradictory reports in the literature on the Zn^{2+} incorporation in HAp; namely about its solubility, ranging from a few % to 15 mol % [17-19], about the Zn atoms location, sorbed on the HAp surface (either six-fold or four-fold coordinated), or incorporated in one of the two crystallographic Ca sites (the nine-fold coordinated Ca1 and the seven-fold coordinated Ca2 sites) of HAp structure [17, 20-28].

2- Materials and methods

2.1 Sol-gel elaboration

The sol-gel route was used to synthesize undoped and Zn-doped HAp samples [16]. Briefly, to produce 2 g of pure HAp powder, 4.7 g of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (Aldrich) and 0.84 g of P_2O_5 (AvocadoResearch chemicals) were dissolved in anhydrous ethanol under stirring and refluxed at 85 °C for 24 h. Then, this solution was kept at 55°C for 24 h, to obtain a consistent gel and further heated at 80°C for 10 h to form powder. Seven samples were prepared from this powder heat treated at 500°C, 600°C, 700°C, 800°C, 900°C, 1000°C and 1100°C during 15 h. To prepare Zn-substituted samples, the required amounts of $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (Acros Organics) was added to the solution, simultaneously with $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$. Nominal compositions have been calculated assuming the previously described interstitial insertion mechanism for zinc into hydroxyapatite: composition $\text{Ca}_{10}\text{Zn}_x(\text{PO}_4)_6\text{O}_{2x}(\text{OH})_{2-2x}$ with a constant Ca/P ratio of 1.67 whatever the Zn content. Four series of samples have been synthesized with $x = 0.00, 0.25, 0.50, 1.00$ (corresponding to nominal ZnO weight percent of 0.0, 2.0, 3.9 and 7.6 wt. % respectively). In the following, samples are labelled $\text{Zn}_x\text{-}y$ with x the amount of Zn^{2+} -doping and y the heat treatment temperature. A total of 28 samples were prepared and analysed.

2.2 X-Ray Powder Diffraction (XRPD)

XRPD patterns were recorded on a X'Pert Pro PANalytical (Almelo, Netherlands) diffractometer, with θ - θ geometry, equipped with a solid detector X-Celerator, and using $\text{CuK}\alpha$ radiation ($\lambda = 1.54184 \text{ \AA}$). XRPD patterns were recorded at room temperature in the interval $3^\circ < 2\theta < 120^\circ$, with a step size of $\Delta 2\theta = 0.0167^\circ$ and a counting time of 200 s for each data value. A total counting time of about 200 min was used for each sample. Figure 1 shows XRPD patterns recorded for the $\text{Zn}_{0.25}$ -series. An XRPD pattern was collected from a pure LaB_6 NIST standard (SRM 660b) by using the same experimental conditions in order to extract the instrumental resolution function to improve the peak profile fitting and to extract intrinsic microstructural parameters.

2.3 Rietveld analyses

Rietveld refinements of X-ray powder patterns were performed for each sample with the program FullProf.2k [29]. The procedure used (both data-collection and refinement strategy) corresponds to the general guidelines for structure refinement using the Rietveld (whole-profile) method formulated by the International Union of Crystallography Commission on Powder Diffraction [30-32]. The initial structural parameters of hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, were taken from [33]: space group $P6_3/m$, $Z = 1$, $a = 9.4218 \text{ \AA}$ and $c = 6.8813 \text{ \AA}$, 7 independent atomic positions: two Ca positions in sites $4f$ ($z = 0.0007$) and $6h$ ($x = 0.2465$, $y = 0.9933$), one P position in site $6h$ ($x = 0.3968$, $y = 0.3693$), and four O positions in sites $6h$ ($x = 0.331$, $y = 0.480$ and $x = 0.579$, $y = 0.455$), $12i$ ($x = 0.3394$, $y = 0.2569$, $z = 0.0694$) and $4e$ ($z = 0.192$ with a half occupancy). Zn atoms were placed in the $2b$ Wyckoff site at $(0, 0, 0)$ [16], after systematic verification of their absence in the Ca1 and Ca2 calcium sites. The initial structural parameters of β -TCP, $\text{Ca}_3(\text{PO}_4)_2$, were taken from [34]: space

group $R3c$, $Z = 21$, $a = 10.4352 \text{ \AA}$ and $c = 37.4029 \text{ \AA}$, 18 independent atomic positions: five Ca positions (three in site $18b$ and two in site $6a$ at one-half occupancy), three P positions (two in site $18b$ and one in site $6a$), and ten O positions (nine in site $18b$ and one in site $6a$). Zincite (ZnO with structural parameters taken from [35]), lime (CaO with structural parameters taken from [36]), and α -TCP ($\text{Ca}_3(\text{PO}_4)_2$ with structural parameters taken from [37]) were observed as minor and temperature dependant phases. The following parameters were first refined: scale factors, zero shift, line profile parameters, lattice parameters, preferential orientations and asymmetry parameters. In a second step atomic displacement factors were refined (only three thermal displacement values were considered - one for divalent cations, one for P and one for O – in order to avoid strong correlation between site occupancies and atomic displacement factors [30-32]), as well as atomic coordinates from the HAp structure. Anisotropic average apparent crystal size parameters were refined in a third step for the HAp phase (refinements were performed with spherical harmonics by using the corresponding $6/m$ Laue class of symmetry). Site occupancies of cations, phosphate and hydroxyl anions were systematically checked in the last runs. Scholzite, $\text{Zn}_2\text{Ca}(\text{PO}_4)_2(\text{H}_2\text{O})_2$, and dehydrated scholzite, $\text{CaZn}_2(\text{PO}_4)_2$ were never observed in any of the 28 samples.

2.4 Micro-Raman spectroscopy

Micro-Raman spectra were recorded at room temperature using a Jobin-Yvon T64000 device. The spectral resolution obtained with an excitation source at 514.5 nm (argon ion laser line, Spectra Physics 2017) is about 1 cm^{-1} . The Raman detector was a charge coupled device (CCD) multichannel detector cooled by liquid nitrogen to 140 K. The laser beam was focused onto the sample through an Olympus confocal microscope with 100x magnifications. Measured power at the sample level was kept low (less than 10 mW) in order to avoid any damage of the material. The Raman scattered light was collected with the microscope

objective at 180° from the excitation and filtered with an holographic Notch filter before being dispersed by a single grating (1800 grooves per mm). Spectra were recorded in the frequencies ranges $100\text{ cm}^{-1} - 1500\text{ cm}^{-1}$ and $3000\text{ cm}^{-1} - 3800\text{ cm}^{-1}$ in order to investigate respectively the vibration modes of phosphate and hydroxyl stretching. Spectra were analysed by a profile fitting procedure using a Lorentzian function.

3- Results

Elemental analysis of the samples by ICP-AES confirms the global nominal compositions as usually observed with this synthesis procedure [38] (Table 1).

3.1 *Mineralogical composition*

Quantitative phase analyses were extracted from Rietveld refinements. Refined mineralogical compositions of the samples are gathered in Table 2. Figure 2 shows the thermal evolution of the HAp, β -TCP, ZnO and CaO weight percents (wt. %). HAp was constantly the major phase. The HAp minimal content is about 65 wt. % observed in $\text{Zn}_{1.00}\text{-900}$. The four series present a similar thermal behaviour: large majority of HAp phase for the sample heat treated at 500°C (99 wt. % for $\text{Zn}_{0.00}\text{-500}$, 98 wt. % for $\text{Zn}_{0.25}\text{-500}$, 97 wt. % for $\text{Zn}_{0.50}\text{-500}$ and 95 wt. % for $\text{Zn}_{1.00}\text{-500}$; i.e. slightly decreasing when increasing the Zn doping level from $\text{Zn}_{0.00}\text{-500}$ to $\text{Zn}_{1.00}\text{-500}$). The weight percent of HAp decreases when temperature increases from 500°C to 900°C (keeping the decreasing sequence from the $\text{Zn}_{0.00}$ -series to the $\text{Zn}_{1.00}$ -series: 80 wt. % for $\text{Zn}_{0.00}\text{-900}$, 77 wt. % for $\text{Zn}_{0.25}\text{-900}$, 70 wt. % for $\text{Zn}_{0.50}\text{-900}$ and 65 wt. % for $\text{Zn}_{1.00}\text{-900}$), then it increases from 900°C to 1100°C to reach almost single phase samples and keeping still the decreasing sequence from the $\text{Zn}_{0.00}$ -series to the $\text{Zn}_{1.00}$ -series (98 wt. % for $\text{Zn}_{0.00}\text{-1100}$, 97 wt. % for $\text{Zn}_{0.25}\text{-1100}$, 95 wt. % for $\text{Zn}_{0.50}\text{-1100}$ and 94 wt. % for $\text{Zn}_{1.00}\text{-1100}$).

In a first step ZnO and CaO wt. % show opposite evolution (Figures 2b and 2c). Between 500°C and 800°C, during the formation of β -TCP, the ZnO content decreases whereas the CaO content increases. In a second step both ZnO and CaO wt. % decrease. One can note the formation of few weight percents of metastable α -TCP at 600°C for all series (5 wt. % for Zn_{0.00}-600, 4 wt. % for Zn_{0.25}-600 and Zn_{0.50}-600, and 3 wt. % for Zn_{1.00}-600) that quickly disappears at 900°C for the Zn_{0.00}-series and already at 700°C for the three Zn-doped series.

3.2 Zn insertion into the HAp structure

Thermal evolution of the HAp lattice parameters evidences the late Zn incorporation into the HAp structure (Figure 3, Table 3). Evolutions of the Zn-doped series start to slightly diverge from the undoped series at 900°C, and are really different at 1000°C and 1100°C. In a first step, from 500°C to 800°C, all the series (undoped and Zn-doped) show a decrease, of about 0.15%, of the unit cell volume (Figure 3c) connected with the increase of crystallinity. Between 800°C and 900°C the undoped series shows a slight increase, of about 0.02%, that could correspond to HAp decarbonation (HAp decarbonation is realized around 900°C [39]). From 900°C, the Zn-doped series differ from undoped series: unit cell volumes of Zn-doped HAp are larger than undoped HAp. More interesting is the lattice parameters evolution: a lattice parameter of Zn-doped HAp is smaller (Figure 3a) whereas c lattice parameter of Zn-doped HAp is larger (Figure 3b) than for undoped HAp. This correlates with previous observation¹⁶ and is attributed to the interstitial mechanism of Zn²⁺ insertion into HAp (in the 2b Wyckoff site). Insertion of small Zn²⁺ cation, contrary to Mg²⁺ case with relatively similar ionic radii [40], induces an increase of the unit cell volume because it inserts the hexagonal channel (creating linear O-Zn-O units oriented along the c hexagonal axis) and it not simply substitutes larger Ca²⁺ cations. We have here a clear indication that cationic size is not the only parameter to consider in order to explain the substitution mechanism into the HAp

structure. Certainly the ability to form linear O-*M*-O ‘complex’ with short *M*-O distances, around 1.7 Å [16], should be considered (Zn²⁺ allows the formation of O-Zn-O entities, whereas slightly smaller Mg²⁺ not).

Refinement of Zn occupancy in the 2*b* Wyckoff site directly confirms the high temperature insertion of Zn into HAp structure (Figure 3d, Table 3). Up to 900°C few Zn insertion is observed: occupancy around 3.5% for the 2*b* Wyckoff site corresponding to the refined Ca₁₀Zn_{0.07}(PO₄)₆(OH)_{1.86}O_{0.14} composition. At 1000°C, the Zn occupancy is about 9%, corresponding to the refined Ca₁₀Zn_{0.18}(PO₄)₆(OH)_{1.64}O_{0.36} composition. And the Zn occupancy reaches about 11% at 1100°C, corresponding to the refined Ca₁₀Zn_{0.22}(PO₄)₆(OH)_{1.56}O_{0.44} composition. The three Zn-doped series show extremely similar behaviours indicating that maximal Zn insertion into HAp structure is already reached for the Zn_{0.25}-series, in agreement with previous result [16] and with mineralogical compositions (excess of zincite in both Zn_{0.50}- and Zn_{1.00}-series).

3.3 Zn substitution in β-TCP

Thermal evolution of β-TCP lattice parameters and unit cell volumes indicates that Zn substitutes calcium during the β-TCP formation; i.e. already at 600°C (Figure 4, Table 4). Unit cell volumes of β-TCP from Zn-doped series are smaller (Figure 4c) than those from the undoped series whatever the heat treatment. The maximum difference between the Zn-doped series and the undoped series is observed between 600°C and 800°C, and then is decreasing. Both *a* and *c* lattice parameters show similar behaviour (Figures 4a and 4b), in agreement with a substitution mechanism of Ca²⁺ by smaller Zn²⁺ and in agreement with previous indication [16]. When β-TCP is destabilized to the benefit of HAp (i.e. above 900°C), Zn is extracted from the β-TCP structure to insert the HAp one (shown by the decrease of the difference between the Zn-doped and the undoped series at 1000°C and 1100°C).

The calcium sites substituted by zinc are temperature dependant (Figure 4d). The two Ca4 and Ca5 sites are subject to Zn substitution; i.e. calcium sites from the low density column described by Yashima et al. [34]. In a first step (at 600°C) Zn fully substitutes calcium in the Ca4 site and is almost not present in the Ca5 site. This trend is totally reversed at 800°C, and returns at 1000°C. The Zn substitution in the Ca4 site lead to a composition of about $\text{Ca}_{2.83}\text{Zn}_{0.17}(\text{PO}_4)_2$ (samples heat treated at 600°C and 1000°C), whereas Zn substitution in the Ca5 site lead to a composition of about $\text{Ca}_{2.67}\text{Zn}_{0.33}(\text{PO}_4)_2$ (samples heat treated at 800°C). The difference in term on Zn incorporation level in β -TCP is not strongly marked in the three Zn-doped series, though refined Zn substitution level is globally increasing when passing from the $\text{Zn}_{0.25}$ -series to the $\text{Zn}_{1.00}$ -series. Despite the homogeneous behaviour on the Zn substitution for the three Zn-doped series (Table 3, Figure 4d), one should take care about the significance of the refined Zn occupancy parameters when β -TCP is present as minor phase: for samples heat treated at 600°C and 1000°C with less than 10 wt % of β -TCP (i.e. when Zn^{2+} cations are observed to substitute exclusively the Ca4 site). Such carefulness is not justified for Zn substitution in the Ca5 site as it concerns samples containing higher quantities of β -TCP (up to 25 wt % for samples heat treated at 800 and 900 °C, Table 3).

3.4 Microstructural effect of Zn

Microstructural parameters have been extracted from Rietveld refinements with anisotropic crystal size for HAp. Crystal sizes for HAp along the $[hh0]$ and the $[00l]$ directions are indicated in Table 3, and isotropic crystal sizes for β -TCP are indicated in Table 4. Both HAp and β -TCP show relatively equivalent average crystal sizes and similar temperature evolutions (increasing from about 200-300 Å at 500°C to about 800 Å at 1100°C). Nevertheless β -TCP shows somewhat smaller crystal size for moderate temperature (up to 800°C). The HAp crystal size anisotropy, calculated by considering the $[00l]/[hh0]$ direction

sizes ratio, is represented in Figure 5. It continuously decreases when increasing the temperature to reach tabular HAp crystals at 1100°C (isotropic morphology with a ratio close to unity). Undoped HAp crystals and Zn-doped HAp crystals showed extremely similar behaviour in term of size and anisotropy.

3.5 *Micro-Raman spectroscopy*

Raman spectra in the range 150 – 1500 cm^{-1} show the four modes of vibration of phosphate tetrahedron. Figure 6 presents the thermal evolution of the ν_1 symmetric stretching mode (the most intense signal) of $[\text{PO}_4]$ for the undoped $\text{Zn}_{0.00}$ - and the Zn-doped $\text{Zn}_{0.25}$ -series. Results from spectral deconvolution are gathered in Table 5 (Raman shift and Full Width at Half Maximum, FWHM). The Raman shift of the ν_1 mode is not temperature dependant for the undoped series, always observed at 963 cm^{-1} . The FWHM is continuously decreasing (from 6.4 to 3.7 cm^{-1}) when increasing the temperature of the heat treatment, indicating the increase of crystallinity. The Zn-doped $\text{Zn}_{0.25}$ -series shows a similar behaviour in a first step (from 500°C to 800°C) with the increase of crystallinity. Then from 900°C to 1100°C the insertion of Zn^{2+} cations into HAp structure was evidenced by the appearance of two new ν_1 components at 959 cm^{-1} and 970 cm^{-1} [16]. The simultaneous increase of the FWHM indicates a loss of order (due to statistic disorder in the hexagonal channel) when Zn^{2+} inserts the HAp crystal structure.

Observations of the $[\text{OH}]$ stretching modes (Figure 7) corroborate those from ν_1 $[\text{PO}_4]$. Increase of the crystallinity from 500°C to 1100°C for the undoped $\text{Zn}_{0.00}$ -series is evidenced by the decrease of FWHM of the band centred at 3572 cm^{-1} (from 10 cm^{-1} to 3 cm^{-1} ; Table 5). A similar behaviour is observed for the $\text{Zn}_{0.25}$ -series up to 800°C, and then the appearance of a new band at 3410 cm^{-1} is attributed to the Zn^{2+} insertion in the $2b$ site. This new band increases in intensity from 900°C to 1100°C, simultaneously with the increase of the FWHM

of the band at 3572 cm^{-1} . This highlights that the HAp crystallinity deterioration (statistic disorder in the hexagonal channel due to partial occupancy of the $2b$ site) is directly correlated to the Zn insertion. Spectra from the $\text{Zn}_{0.25}$ -1100 sample show the presence of a third [OH] stretching mode at 3584 cm^{-1} (shoulder on the main 3572 cm^{-1} component), certainly also correlated to the Zn insertion.

4- Discussion

During heat treatment, a partial and reversible transformation of HAp into β -TCP is observed (Figure 2a). β -TCP is completely absent in samples heat treated at 500°C , and is almost absent in samples heat treated at 1100°C (between 1.5 and 3.5 wt. %). The almost single phase feature of samples heat treated at 1100°C definitively strengthens the insertion mechanism for Zn (by validating the used constant Ca/P ratio of 1.67 whatever the Zn content). The β -TCP phase reaches its maximal concentration at 900°C (19 wt. % for $\text{Zn}_{0.00}$ -900, 21 wt. % for $\text{Zn}_{0.25}$ -900, 26 wt. % for $\text{Zn}_{0.50}$ -900 and 28 wt. % for $\text{Zn}_{1.00}$ -900). Thermal evolution of HAp lattice parameters (Figure 3) clearly highlights that Zn inserted the HAp structure from 1000°C only, whereas it substitutes the β -TCP structure already at 600°C (the temperature of the β -TCP formation; Figure 4). Only weak Zn incorporation in HAp is realized before 1000°C . Such a high temperature for Zn insertion in HAp can explain the divergences founded in the literature concerning the mechanism of Zn incorporation [16,17,20-23]. The similar thermal evolution of lattice parameters of the undoped and the Zn-doped series before 900°C indicates that almost no Zn incorporates the HAp structure up to 800°C . Only Zn physisorbed at the HAp surface could be envisaged (as proposed by Bazin et al. [25] and Bigi et al. [19]). Raman spectra fully agree with the late Zn insertion into the HAp structure: both $[\text{PO}_4]$ and [OH] stretching modes indicate a structural modification from 900°C only (Figures 5 and 6). This new understanding of the high temperature mechanism of

Zn^{2+} incorporation in HAp lattice explains previously published results on related materials such as the crystallinity dependence of the maximum zinc content in HAp or the crystallinity dependence of the bone formation promotion [6]. It also clearly demonstrates that precipitation of a Zn-containing apatite layer, as observed by wang et al. [9], is not a layer constituted of a Zn-doped HAp compound.

The β -TCP content increase from $\text{Zn}_{0.00}$ -series to $\text{Zn}_{1.00}$ -series, observed whatever the temperature, results in the late Zn incorporation into HAp. The fact that Zn-insertion in HAp is not realized for the moderate temperatures is at the origin of the β -TCP-stabilizing feature of zinc described in the literature [19,22,41]. Zn-doped β -TCP crystals were somewhat smaller than those from undoped series (Table 4), and Zn-doped HAp crystal anisotropy of morphology was somewhat smaller than the one for undoped series (Figure 5). Such weak differences between the undoped and the Zn-doped series are contradictory with previously reported inhibiting effect of zinc on HAp crystallisation and the preference of Zn for β -TCP [17,41,42]. Present results clearly evidence that the Zn-stabilizing feature is temperature-dependant (Zn stabilizes β -TCP below 900°C and stabilizes HAp above 900°C). The Zn incorporation into β -TCP with a Ca/P ratio of 1.5 induces the formation of CaO around 900°C (Figure 2c). The following Zn incorporation into HAp (from 900°C) with a Ca/P ratio of 1.67, the nominal one, explains the complete disappearance of CaO in the four samples heat treated at 1100°C (Table 2, Figure 2c).

Samples heat treated at 500°C, in which HAp (that do not yet incorporate Zn, or weakly) is the only calcium phosphate phase, contain about half of the zincite, ZnO, nominal content only (1.2 wt. % against the nominal 2.0 wt. % for the $\text{Zn}_{0.25}$ -series, 2.2 wt. % against the nominal 3.9 wt. % for the $\text{Zn}_{0.50}$ -series, and 4.5 wt. % against the nominal 7.6 wt. % for the $\text{Zn}_{1.00}$ -series). This indicates that about half of the incorporated Zn atoms were not detectable by XRPD. They are either in an amorphous compound and/or physisorbed at the surface of

HAp. This situation persists up to 800°C in a more pronounced manner due to Zn incorporation into β -TCP, next the weight percent of ZnO phase increases to reach a maximum at 900-1000°C (when β -TCP is destabilized to the profit of HAp that does not yet fully incorporate Zn). The ZnO content observed in samples heat treated at 1100°C (almost absent in Zn_{0.25}-1100, 1.1 wt. % in Zn_{0.50}-1100, and 4.8 wt. % in Zn_{1.00}-1100) traduces the fact that HAp is not allowed to incorporate all the nominal Zn content for the Zn_{0.50}- and Zn_{1.00}-series. For the three Zn-doped samples heat treated at 1100°C, about 2 wt. % of ZnO is inserted into HAp corresponding to the composition Ca₁₀Zn_{0.25}(PO₄)₆(OH)_{1.5}O_{0.5}; close to the refined composition of the Zn-HAp crystallographic structure previously described [16]. The formation of an interstitial solid solution is evidenced by refining the Zn occupancy in the 2*b* Wyckoff site, and is also clearly evidenced by the anisotropic evolution of the HAp lattice parameters: *a* lattice parameter is decreasing whereas *c* lattice parameter is increasing when entering small Zn²⁺ cations. The *x* = 0.25 limit value corresponds to 25% of the Ca₁₀Zn(PO₄)₆O₂ composition allowed by electroneutrality consideration only. When considering Zn atom located in HAp, β -TCP and ZnO for all temperature it clearly appears that from about 800°C all the nominal Zn content is detectable by XRPD (Figure 8). This is not the case between 500°C and 700°C. A part of Zn is either contained in an amorphous compound and/or physisorbed at the HAp surface. At 600°C the divergence reaches its maximal value for the three series, but presence of α -TCP (metastable at this temperature [43]) that can incorporate Zn should be considered (i.e. refined Zn content is underestimated at 600°C). One can remark that the higher the Zn nominal content, the higher the divergence. The undetectable Zn content highly increases from the Zn_{0.25}-series (for which total refined Zn content continuously agrees relatively well with the targeted value of 1.60 wt. %) to the Zn_{1.00}-series. The part of Zn atoms that is not located below 800°C, followed by the Zn transfer from β -TCP to HAp between 800°C and 1000°C, could explain the contradictions in

the literature in term of Zn environments. The Zn environments in samples heat treated at 500°C (in amorphous compound, or probably physisorbed at the HAp surface), is clearly not the same that those from samples heat treated at 700-900°C (with the presence of Zn-substituted β -TCP), and not the same as those from samples heat treated at 1000-1100°C (with formation of linear O-Zn-O entities in HAp structure).

5- Conclusion

Rietveld analyses performed on XRPD patterns from undoped and Zn-doped HAp samples heat treated between 500°C and 1100°C strengthen and explicit the mechanism of Zn insertion in the interstitial $2b$ Wyckoff position of the HAp structure previously described and corresponding to the general $\text{Ca}_{10}\text{Zn}_x(\text{PO}_4)_6(\text{OH})_{2-2x}\text{O}_{2x}$ composition [16]. For moderate heat treatment temperature, Zn stabilizes the β -TCP phase by substituting calcium in the low density column of the β -TCP structure (i.e. Ca4 and Ca5 sites). Up to 800°C weak quantity of Zn enters the interstitial hexagonal channel of the HAp phase; $\text{Ca}_{10}\text{Zn}_x(\text{PO}_4)_6(\text{OH})_{2-2x}\text{O}_{2x}$ with a x value around 0.07 only. At 800°C-900°C about 25 wt. % of β -TCP is observed (with about 2 wt. % of joint CaO phase due to the nominal Ca/P ratio of 1.67). The Zn quantity inserted at this stage in β -TCP leads to the composition $\text{Ca}_{2.67}\text{Zn}_{0.33}(\text{PO}_4)_2$. Above 900°C, Zn inserted in larger extent the hexagonal channel of HAp structure, and consequently destabilizes the β -TCP phase (by consuming the CaO phase formed at intermediate temperatures). The Zn-doped HAp phase reaches a composition close to $\text{Ca}_{10}\text{Zn}_{0.25}(\text{PO}_4)_6(\text{OH})_{1.5}\text{O}_{0.5}$, $\text{Ca}_{10}\text{Zn}_x(\text{PO}_4)_6(\text{OH})_{2-2x}\text{O}_{2x}$ with $x = 0.25$, which appears to be the maximal value of zinc insertion into HAp. The high temperature insertion of Zn into HAp stabilizes the β -TCP phase at moderate temperature and is certainly at the origin of the reported inhibiting effect of zinc on HAp crystallisation and the preference of Zn for β -TCP [17,41,42], which are not at all maintained at 1000°C and 1100°C. The environment of Zn atoms (without considering the

excess contained in zincite for the two $\text{Zn}_{0.50}$ - and $\text{Zn}_{1.00}$ -series) is temperature dependant because Zn moves from a non-definite position at 500°C (in an amorphous compound or physisorbed at HAp surface), to a partial substitution in the low-density column of the β -TCP structure at around 800°C , and finally to the insertion in the hexagonal channel of the HAp structure (in the $2b$ site, and forming linear O-Zn-O entities). Such a temperature dependent mechanism for Zn incorporation into HAp material should be considered by researchers when working on the biological effects of Zn-containing calcium phosphate materials. In particular, material preparation method should be clearly indicated and careful characterization should be carried out.

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Table 1. Nominal and experimental (determined by ICP-AES with standard deviations indicated) compositions for the four synthesized series.

	Nominal targeted composition					Experimental composition				
	CaO (wt %)	P ₂ O ₅ (wt %)	Ca/P	ZnO (wt %)	x ^a	CaO (wt %)	P ₂ O ₅ (wt %)	Ca/P	ZnO (wt %)	x ^a
Zn _{0.00} -series	56.84	43.16	1.67	-	0.00	57 ± 1	43.1 ± 0.9	1.67 ± 0.04	-	0.00 (-)
Zn _{0.25} - series	55.69	42.29	1.67	2.00	0.25	56 ± 1	42.7 ± 0.9	1.67 ± 0.04	2.0 ± 0.1	0.25 ± 0.01
Zn _{0.50} - series	54.59	41.45	1.67	3.93	0.50	55 ± 1	41.2 ± 0.8	1.69 ± 0.04	4.0 ± 0.2	0.51 ± 0.03
Zn _{1.00} - series	52.51	39.87	1.67	7.62	1.00	53 ± 1	40.4 ± 0.8	1.68 ± 0.04	7.6 ± 0.4	0.98 ± 0.05

^a x value from the global composition Ca₁₀Zn_x(PO₄)₆O_{2x}(OH)_{2-2x}.

Table 2. Results of the quantitative analyses (wt. %) extracted from Rietveld refinements. Standard deviations, corresponding to σ values given by FullProf output files, are indicated in parentheses.

series	samples	Mineralogical composition (wt. %)				
		HAp	β -TCP	α -TCP	ZnO	CaO
Zn _{0.00} -series	Zn _{0.00} -500	99.4 (3)	-	-	-	0.64 (2)
	Zn _{0.00} -600	91.5 (3)	3.0 (1)	4.7 (1)	-	0.78 (2)
	Zn _{0.00} -700	86.8 (3)	7.9 (1)	4.2 (1)	-	1.10 (2)
	Zn _{0.00} -800	81.7 (3)	14.3 (2)	2.8 (1)	-	1.18 (3)
	Zn _{0.00} -900	80.3 (3)	18.8 (2)	-	-	0.89 (2)
	Zn _{0.00} -1000	89.0 (3)	10.6 (1)	-	-	0.38 (2)
	Zn _{0.00} -1100	97.5 (4)	2.51 (8)	-	-	-
Zn _{0.25} - series	Zn _{0.25} -500	98.3 (3)	-	-	1.18 (2)	0.51 (2)
	Zn _{0.25} -600	88.5 (4)	6.4 (2)	3.8 (1)	0.71 (2)	0.64 (2)
	Zn _{0.25} -700	82.1 (4)	16.3 (3)	-	0.51 (2)	1.09 (2)
	Zn _{0.25} -800	78.0 (3)	20.0 (2)	-	0.56 (2)	1.43 (3)
	Zn _{0.25} -900	76.7 (3)	21.3 (2)	-	0.66 (2)	1.29 (2)
	Zn _{0.25} -1000	82.3 (3)	11.7 (2)	-	0.43 (2)	0.60 (2)
	Zn _{0.25} -1100	96.5 (3)	3.46 (9)	-	0.16 (2)	-
Zn _{0.50} - series	Zn _{0.50} -500	97.3 (3)	-	-	2.17 (5)	0.56 (2)
	Zn _{0.50} -600	87.3 (4)	6.8 (3)	3.6 (1)	1.51 (2)	0.75 (2)
	Zn _{0.50} -700	82.2 (4)	15.3 (2)	-	1.33 (2)	1.26 (2)
	Zn _{0.50} -800	74.1 (3)	22.7 (2)	-	1.53 (3)	1.67 (2)
	Zn _{0.50} -900	70.1 (2)	26.4 (2)	-	1.95 (2)	1.50 (2)
	Zn _{0.50} -1000	79.7 (3)	17.5 (2)	-	2.33 (2)	0.45 (2)
	Zn _{0.50} -1100	95.4 (4)	3.1 (1)	-	1.52 (2)	-
Zn _{1.00} - series	Zn _{1.00} -500	95.2 (4)	-	-	4.48 (7)	0.37 (2)
	Zn _{1.00} -600	81.5 (5)	10.6 (4)	3.34 (2)	3.42 (3)	1.12 (2)
	Zn _{1.00} -700	74.5 (4)	19.5 (2)	-	3.66 (4)	2.37 (3)
	Zn _{1.00} -800	68.4 (3)	24.7 (2)	-	4.35 (3)	2.50 (3)
	Zn _{1.00} -900	64.6 (2)	27.9 (2)	-	5.40 (3)	2.12 (2)
	Zn _{1.00} -1000	75.4 (3)	17.6 (2)	-	5.91 (3)	1.00 (2)
	Zn _{1.00} -1100	93.5 (4)	1.27 (8)	-	5.24 (4)	-

Table 3. HAp structural and microstructural parameters extracted from Rietveld refinements.

Standard deviations, corresponding to σ values given by FullProf output files, are indicated in parentheses.

series	samples	HAp				Crystal size	
		a (Å)	c (Å)	V (Å ³)	Zn (%) [*]	$[hh0]$ (Å)	$[00l]$ (Å)
Zn _{0.00}	Zn _{0.00} -500	9.4263 (1)	6.8815 (1)	529.53 (1)	-	240	310
	Zn _{0.00} -600	9.4190 (1)	6.88315 (8)	528.85 (1)	-	260	320
	Zn _{0.00} -700	9.41782 (7)	6.88401 (6)	528.778 (7)	-	310	370
	Zn _{0.00} -800	9.4182 (1)	6.8825 (1)	528.70 (1)	-	450	500
	Zn _{0.00} -900	9.42052 (3)	6.88127 (3)	528.870 (3)	-	620	670
	Zn _{0.00} -1000	9.42048 (2)	6.88158 (2)	528.889 (3)	-	820	850
	Zn _{0.00} -1100	9.42016 (2)	6.88203 (2)	528.888 (2)	-	910	930
Zn _{0.25}	Zn _{0.25} -500	9.4235 (1)	6.8845 (1)	529.46 (1)	3.6 (3)	260	320
	Zn _{0.25} -600	9.4166 (1)	6.8853 (1)	528.74 (1)	3.0 (3)	290	350
	Zn _{0.25} -700	9.41629 (8)	6.88425 (7)	528.624 (8)	3.0 (3)	320	370
	Zn _{0.25} -800	9.41864 (6)	6.88276 (5)	528.773 (6)	3.0 (3)	470	490
	Zn _{0.25} -900	9.41854 (4)	6.88548 (4)	528.971 (5)	5.4 (3)	630	650
	Zn _{0.25} -1000	9.41717 (3)	6.89363 (2)	529.444 (3)	9.6 (3)	770	780
	Zn _{0.25} -1100	9.41272 (2)	6.90081 (2)	529.494 (2)	10.8 (3)	860	870
Zn _{0.50}	Zn _{0.50} -500	9.4228 (1)	6.8849 (1)	529.41 (1)	3.6 (3)	270	340
	Zn _{0.50} -600	9.4173 (1)	6.8857 (1)	528.85 (1)	4.2 (3)	300	370
	Zn _{0.50} -700	9.41654 (9)	6.88526 (8)	528.730 (9)	3.6 (3)	330	390
	Zn _{0.50} -800	9.41815 (7)	6.88370 (3)	528.791 (8)	3.6 (3)	480	510
	Zn _{0.50} -900	9.41909 (5)	6.88605 (4)	529.077 (5)	5.4 (3)	600	620
	Zn _{0.50} -1000	9.41665 (3)	6.89355 (3)	529.378 (3)	9.0 (3)	760	770
	Zn _{0.50} -1100	9.41153 (4)	6.90100 (3)	529.375 (4)	11.2 (3)	870	870
Zn _{1.00}	Zn _{1.00} -500	9.4242 (2)	6.8863 (1)	529.68 (2)	4.2 (3)	270	340
	Zn _{1.00} -600	9.4170 (1)	6.88611 (9)	528.85 (1)	4.8 (3)	290	360
	Zn _{1.00} -700	9.41677 (8)	6.88440 (7)	528.535 (9)	3.0 (3)	400	470
	Zn _{1.00} -800	9.41833 (7)	6.88249 (6)	528.718 (8)	3.6 (3)	520	550
	Zn _{1.00} -900	9.41882 (5)	6.88520 (4)	528.981 (5)	4.2 (3)	620	650
	Zn _{1.00} -1000	9.41762 (3)	6.89376 (3)	529.504 (3)	8.4 (3)	760	770
	Zn _{1.00} -1100	9.41382 (4)	6.90157 (4)	529.676 (4)	12.6 (3)	880	880

^{*} Occupancy of Zn in the 2*b* Wyckoff site.

Table 4. β -TCP structural and microstructural parameters extracted from Rietveld refinements. Standard deviations, corresponding to σ values given by FullProf output files, are indicated in parentheses.

series	samples	β -TCP					Crystal size (Å)
		a (Å)	c (Å)	V (Å ³)	Zn^*		
					Ca4 (%)	Ca5 (%)	
$Zn_{0.00}$	$Zn_{0.00}$ -500	-	-	-	-	-	-
	$Zn_{0.00}$ -600	10.444 (1)	37.382 (5)	3531.2 (7)	-	-	380
	$Zn_{0.00}$ -700	10.4394 (4)	37.383 (2)	3528.2 (3)	-	-	420
	$Zn_{0.00}$ -800	10.4367 (5)	37.389 (2)	3527.0 (3)	-	-	540
	$Zn_{0.00}$ -900	10.4367 (1)	37.3907 (5)	3527.10 (7)	-	-	680
	$Zn_{0.00}$ -1000	10.4349 (2)	37.3889 (8)	3525.8 (1)	-	-	790
	$Zn_{0.00}$ -1100	10.4309 (5)	37.385 (1)	3522.6 (2)	-	-	960
$Zn_{0.25}$	$Zn_{0.25}$ -500	-	-	-	-	-	-
	$Zn_{0.25}$ -600	10.380 (1)	37.255 (5)	3476.2 (7)	50 (-)	0 (-)	210
	$Zn_{0.25}$ -700	10.3573 (5)	37.268 (2)	3462.3 (3)	31 (6)	58 (5)	230
	$Zn_{0.25}$ -800	10.3717 (2)	37.2670 (8)	3471.8 (1)	8 (1)	74 (3)	320
	$Zn_{0.25}$ -900	10.3974 (2)	37.3209 (7)	3494.1 (1)	8 (1)	50 (3)	610
	$Zn_{0.25}$ -1000	10.4112 (2)	37.3630 (9)	3507.3 (1)	50 (-)	10 (5)	670
	$Zn_{0.25}$ -1100	10.4173 (8)	37.375 (3)	3512.6 (5)	-	-	820
$Zn_{0.50}$	$Zn_{0.50}$ -500	-	-	-	-	-	-
	$Zn_{0.50}$ -600	10.355 (2)	37.213 (8)	3456 (1)	50 (-)	0 (-)	140
	$Zn_{0.50}$ -700	10.3431 (5)	37.230 (2)	3449.3 (3)	39 (7)	36 (6)	230
	$Zn_{0.50}$ -800	10.3493 (2)	37.2582 (8)	3456.0 (1)	4 (1)	94 (3)	350
	$Zn_{0.50}$ -900	10.3868 (2)	37.2955 (7)	3484.6 (1)	8 (1)	62 (2)	700
	$Zn_{0.50}$ -1000	10.4148 (1)	37.3680 (6)	3510.23 (9)	50 (-)	20 (3)	720
	$Zn_{0.50}$ -1100	10.4181 (9)	37.360 (4)	3511.7 (5)	-	-	840
$Zn_{1.00}$	$Zn_{1.00}$ -500	-	-	-	-	-	-

Zn _{1.00} -600	10.330 (2)	37.21 (1)	3439 (1)	50 (-)	13 (9)	180
Zn _{1.00} -700	10.3332 (2)	37.224 (1)	3442.1 (1)	19 (5)	100 (-)	380
Zn _{1.00} -800	10.3441 (2)	37.2296 (8)	3449.9 (1)	4 (1)	100 (-)	490
Zn _{1.00} -900	10.3847 (2)	37.2957 (6)	3483.19 (9)	7 (1)	73 (4)	690
Zn _{1.00} -1000	10.4141 (2)	37.3704 (6)	3509.99 (9)	40 (4)	24 (3)	690
Zn _{1.00} -1100	10.418 (1)	37.355 (5)	3511.1 (7)	-	-	780

* Occupancies of Zn in the Ca4 and Ca5 calcium sites from β -TCP structure.³⁴

Table 5. Raman spectra decomposition of the Zn_{0.00}- and Zn_{0.25}-series. Raman shift and full width at half maximum (FWHM, in parentheses) for symmetric stretching mode of phosphate tetrahedron and hydroxyl stretching.

Temperature	Zn _{0.00} -series		Zn _{0.25} -series	
	ν_1 [PO ₄] (cm ⁻¹)	[OH] stretching (cm ⁻¹)	ν_1 [PO ₄] (cm ⁻¹)	[OH] stretching (cm ⁻¹)
500 °C	963 (6.4)	3572 (10.1)	963 (7.7)	3572 (12.3)
600 °C	963 (6.3)	3572 (8.7)	963 (6.2)	3572 (10.3)
700 °C	964 (6.1)	3572 (8.4)	963 (5.8)	3572 (7.5)
800 °C	963 (5.1)	3572 (5.4)	963 (4.9)	3572 (5.0)
900 °C	963 (4.8)	3572 (4.2)	963 (5.6)	3410 (26.6) 3572 (7.1)
1000 °C	963 (3.7)	3572 (2.9)	959 (6.1) [*] 963 (6.1) [*] 970 (6.1) [*]	3411 (25.4) 3572 (8.4)
1100 °C	963 (3.7)	3572 (3.0)	959 (6.3) [*] 964 (6.3) [*] 970 (6.3) [*]	3412 (25.3) 3572 (8.8) 3584 (1.2)

^{*} FWHM of the three ν_1 components were constraint to be equal during the deconvolution procedure.

Figure 1. PXRD patterns for the $\text{Zn}_{0.25}$ -series ($\lambda = 1.54184 \text{ \AA}$). Major phase is HAp for all patterns, minor phases are ZnO, α -TCP, CaO and β -TCP (for which main diffraction peaks are respectively marked by +, *, ", and °).

Figure 2. Quantitative phases analyses extracted from Rietveld refinement (a: HAp and β -TCP phases, b: ZnO, and c: CaO) performed for the undoped $\text{Zn}_{0.00}$ -series (open squares symbols, dashed lines), and the Zn-doped $\text{Zn}_{0.25}$ -series (full circles), $\text{Zn}_{0.50}$ -series (full diamonds), $\text{Zn}_{1.00}$ -series (full stars).

Figure 3. Thermal evolutions of the HAp lattice parameters (a: parameter a , b: parameter c), the HAp unit cell volume (c), and the Zn occupancy factor in the $2b$ Wyckoff site (d) for the undoped $\text{Zn}_{0.00}$ -series (open squares symbols, dashed lines), and the Zn-doped $\text{Zn}_{0.25}$ -series (full circles), $\text{Zn}_{0.50}$ -series (full diamonds), $\text{Zn}_{1.00}$ -series (full stars). Error bars correspond to 3σ (within symbols for lattice parameters and unit cell volumes).

Figure 4. Thermal evolutions of the β -TCP lattice parameters (a: parameter a , b: parameter c), and the β -TCP unit cell volume (c) for the undoped $\text{Zn}_{0.00}$ -series (open squares symbols, dashed line), and the Zn-doped $\text{Zn}_{0.25}$ -series (full circles), $\text{Zn}_{0.50}$ -series (full diamonds), $\text{Zn}_{1.00}$ -series (full stars). Ca substitution percent is represented (d) in site Ca4 (full symbols) and in site Ca5 (open symbols, dotted lines). Error bars correspond to 3σ .

Figure 5. HAp crystal size anisotropy ($[00l]/[hh0]$ direction sizes ratio) for the undoped $\text{Zn}_{0.00}$ -series (open squares symbols, dashed line), and the Zn-doped $\text{Zn}_{0.25}$ -series (full circles), $\text{Zn}_{0.50}$ -series (full diamonds), $\text{Zn}_{1.00}$ -series (full stars).

Figure 6. Details of the Raman spectra showing the thermal evolution of the ν_1 mode of vibration of $[\text{PO}_4]$ for the undoped $\text{Zn}_{0.00}$ -series (left) and the Zn-doped $\text{Zn}_{0.25}$ -series (right).

Figure 7. Raman spectra showing the $[\text{OH}]$ vibrations for the undoped $\text{Zn}_{0.00}$ -series (left) and the Zn-doped $\text{Zn}_{0.25}$ -series (right).

Figure 8. Total refined Zn content (from the three Zn-doped HAp, Zn-doped β -TCP and ZnO phases) for the Zn-doped $\text{Zn}_{0.25}$ -series (full circles), $\text{Zn}_{0.50}$ -series (full diamonds), $\text{Zn}_{1.00}$ -series (full stars). Dotted horizontal lines correspond to nominal targetted values: respectively 1.6, 3.2 and 6.1 wt. %.

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